

Fig. 3 shows percent inhibition as a function of competing fibrinogen concentration (Example 1C),

Fig. 4 shows the protease sensitivity of adherence to fibrinogen (Example 1D),

Fig. 5 shows the inhibition of adherence by LiCl extract (Example 1E),

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| Figures 6A-6E show the complete nucleotide sequence of the *fig* gene from *S. epidermidis* strain HB and the deduced amino acid sequence of the encoded protein (SEQ ID NO:14). A putative ribosomal binding site (RBS) is underlined and a possible transcription termination hairpin loop is double underlined. A putative signal sequence (S) is indicated with an arrow and the translational stop codon with an asterisk. The start of the non-repetitive N-terminal region called A, harbouring the fibrinogen binding activity-is indicated by an arrow. R indicates the highly repetitive region. The motif LPXTG involved in cell wall anchoring is indicated in bold characters and the membrane-spanning region is marked M (Example 3 ),

Seq. non-coupl

Fig. 7 shows a schematic drawing comparing the fibrinogen binding protein FIG of *S. epidermidis* and the clumping factor (ClfA) of *S. aureus*. The similarity, (%), of corresponding regions in the proteins is indicated in the figure between the two protein bars. S is the signal sequence; A, the non-repetitive region harbouring the fibrinogen binding activity, R, the diamino acid residue repeat region; W the region proposed to be involved in cell wall anchoring and M, the transmembrane domain. The numbers indicated refer to the amino acid positions in respective proteins as shown in figures 6A-6E and 7 and in reference (McDevitt et al., 1994) (Example 3),

Fig. 8 shows how GST-FIG fusion protein is captured to fibrinogen in a dose dependent way (Example 10),

Fig. 9 shows the decrease of bacterial binding as a function of GST-FIG fusion protein, GST or FIG (Example 11),

Fig. 10 shows the relative adherence as function of serum dilution for two pre immune sera and a serum against GST-FIG and FIG, respectively (Example 12), and

Fig. 11 shows the relative bacterial adherence as a function of serum dilution for, on one hand, pre immune serum and, on the other hand, serum against GST-FIG (Example 12).

Please replace the paragraph on page 7, beginning at line 8 and ending at line 21, with the following rewritten paragraph:

Fibrinogen was dissolved in PBS at 10 mg/ml and added in serial 3-fold dilution to microtiter wells (Nunc), from top to bottom. The plates were incubated overnight at room temperature (RT). To cover uncoated plastic sites the plates were coated with 2% bovine serum albumin for 1 hour at 37°C. The plates were washed with PBS with 0.05% TWEEN 20 (PBST). Next, bacteria were added in serial 2-fold dilution in PBST, from left to right, to the fibrinogen coated microtiter plates. Bacterial adherence was allowed for 2 hours at 37°C or at 4°C overnight. Non-adherent bacteria were washed off and the bound bacteria were air-dried. The crosswise dilution of both fibrinogen and bacteria allows estimation of bacterial binding both as a function of fibrinogen concentration and of amount of bacteria. Determination of bacterial adherence was done by optical reading using a microtiter plate reader at A 405. The turbidity and light scatter caused by bound bacteria results in a reading ranging from 0.00 to 0.20. An example of adherence values as a function of fibrinogen coating concentration is shown in Figure 1 for three different strains (2, 19 and JW27). These conditions for adherence determination were used in the following experiments.

Please replace the paragraph on page 10, beginning at line 6 and ending at line 29, with the following rewritten paragraph:

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To obtain the missing 5' and 3' end of the *fig* gene a Southern blot analysis was performed using chromosomal DNA from strain HB digested with various restriction enzymes. The probe was prepared as follows; two oligonucleotides (5'CAACAACCATCTCACACAAC3' which is SEQ ID NO:1 and 5'CATCAAATTGATATTTCCCATC3' which is SEQ ID NO:2) were used to PCR amplify a ~1.3kb fragment from the insert of pSE100. The PCR generated fragments were 32P-labeled using random priming. After hybridisation using stringent conditions the NC-filter was washed and subjected to autoradiography. The result showed that the XbaI cleavage gave a single band in size of ~6 kb. The corresponding fragment was subsequently ligated into XbaI digested pUC18 vector. After transformation clones harbouring the ~6kb XbaI-fragment were identified by colony hybridisation using the same probe as in the Southern blot experiment. One such clone, called pSE101 was chosen for further studies. DNA sequence analysis showed that the *fig* gene consist of an open reading frame of a 3291 nt, encoding a protein, called FIG of 1097 aa with a calculated molecular mass of ~119 kDa (figures 6A-6E). The FIG protein consist of several typical features found among Gram-positive cell surface bound proteins, like a N-terminal signal sequence and a C-terminal aa motif LPDTG, followed by a stretch of 17 hydrophobic aa ending in a stretch of charged aa (Figure 6). Following the signal sequence, there is a region, called A of 773 aa. The insert of pSE100 contains the sequence corresponding to residue 75 to 656 of the A region (Figure. 7). The A region is followed by a highly repetitive region of 216 aa composed of tandemly repeated aspartic acid and secine residues, called R (figures 6A-6E and 7). The dipeptid region consist of an 18 bp sequence unit (consensus of GAX TCX GAX TCX GAX AGX which is SEQ ID NO:3) repeated 36 times. The 18 bp sequence is almost maintained perfect throughout the whole R region except for the second unit which is truncated,

seq. not complete

Serial No. 09/147,406

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cont consisting of only 12 of the 18 bp and the 3' end of the region where the consensus sequence is slightly disrupted (units 32, 34 and 36). The changes in the later units also result in an amino acid exchange which disrupt the DS repeat.

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Please replace the paragraph on page 13, beginning at line 12 and ending at page 14, line 5, with the following rewritten paragraph:

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Example 5: Western blot experiment

24 *E. coli* cells of strain TG1 and MC1061 containing pSE100 were grown in LB (containing ampicillin and glucose) over night at 37°C. The next morning the cells were harvested by centrifugation, resuspended in LB (containing ampicillin, glucose and 0.1 M IPTG and further incubated at 37°C. Twelve hours later the cells were harvested by centrifugation and both the cells and the supernatant were taken care of. Four volumes of acetone were added to the supernatant and the resulting precipitate was collected by centrifugation, air-dried and resuspended in ice-cold PBS. Prior to electrophoresis the cells and the precipitate from the supernatant were resuspended separately in a sample buffer containing 2.5% SDS and 5% beta-mercaptoethanol and boiled for two minutes. After denaturation the samples were analysed run under reducing conditions using the PHAST-system (Pharmacia) on a 8-25% gradient gel using SDS-buffer strips. After the electrophoresis was completed a NC-filter previously soaked in PBS was put on the gel and the temperature raised to 45°C. After ~45 minutes the NC-filter was wetted with 1 ml PBS, gently removed and placed in 15 ml PBS containing 0.1% TWEEN 20 solution (PBST 0.1%) for 30 minutes in RT (under gentle agitation and with two changes of PBST 0.1% solution). After the last change of PBST 0.1% fibrinogen was added to a final conc. of 20ng/ml and the filter was incubated for four hours at RT under gentle agitation. The filter was subsequently washed for 3x10 minutes using PBST 0.1% and HRP-conjugated rabbit anti-human fibrinogen antibodies (DAKO code A 080, diluted 1:500 in PBST 0.1%)

Serial No. 09/147,406

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were added and the filter was incubated for 1 hour at RT under gentle agitation. After washing the filter 3x10 minutes using PBST 0.1 % the bound fibrinogen was visualised by transferring the filter to a solution containing a substrate for the horse radish peroxidase (6 ml 4-chloro-1-naphtol (3 mg/ml in methanol) + 25 ml PBS + 20  $\mu$ l  $H_2O_2$ ). The result showed that a fibrinogen binding protein was found in both types of samples (cells and growth media) in both *E. coli* cells harbouring pSE100, while no such protein was found in the control cultures of *E. coli* TG1 and MC1061. The fibrinogen binding protein expressed from the pSE100 was in the approximate size as expected from the deduced amino acid.

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Please replace the paragraph on page 16, beginning at line 1 and ending at line 17, with the following rewritten paragraph:

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#### Example 9: Production of GST-FIG

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By polymerase chain reaction, a DNA fragment was amplified encoding a portion of the fibrinogen binding protein. Upper primer was GCGGATCCAATCAGTCAATAAACACCGACGAT (SEQ ID NO:8) and lower primer was CGGAATTCTGTTCGGACTGATTTGGAAGTTCC (SEQ ID NO:9). Amplification was done for 30 cycles at 94°C 30 seconds, 60°C 30 seconds, 72°C 2 minutes beginning with 94°C for 4 minutes and ending with 72°C for 4 minutes. The amplified fragment was digested with EcoRI and Bam HI. Plasmid pGXT-4T (Pharmacia, Uppsala, Sweden) was digested with EcoRI and Barn HI, mixed with the digested fragment and the mixture ligated using T4 DNA ligase according to standard procedures. The ligated DNA was transformed into *E. coli* strain TGI. A transformant was isolated with a plasmid encoding a fusion protein composed of glutathione thio transferase and fibrinogen binding protein. The protein was purified with the vector plasmid according to Pharmacia's instructions. The purified GST-FIG protein was subjected to Western affinity blot. It was run on polyacrylamide gel electrophoresis,

Serial No. 09/147,406

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transferred to nitrocellulose paper by passive diffusion, the paper treated with fibrinogen (5  $\mu$ g/ml) for 2 hours at room temperature, followed by rabbit anti fibrinogen antibodies conjugated to HRP. A band corresponding to a molecular weight of approx. 100 kDa was seen. Omitting fibrinogen in a control experiment displayed no band.

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